Autoimmune Technologies LLC

Applied Biomedical Science

ANTIBODIES LINK GULF WAR SYNDROME TO ANTHRAX VACCINE

FOR IMMEDIATE RELEASE

New Orleans, July 15, 2002 - There is new immunological evidence that a contaminant in certain lots of the Department of Defense's anthrax vaccine may be the cause of Gulf War Syndrome.

Gulf War Syndrome, or GWS, is an illness that was first described in veterans of the 1990-1991 Persian Gulf war. Symptoms include muscle aches and joint pain, chronic fatigue, headaches, anxiety, depression, dizziness, sleep disorders, rashes, and loss of concentration. Cases of a similar illness have been seen in personnel who were not deployed to the Persian Gulf theater of operations in 1990 or 1991 and also in personnel who joined the armed forces after 1991, though the illness in these patients has not been called GWS.

Data published in an article entitled "Antibodies to Squalene in Recipients of Anthrax Vaccine" in the August 2002 issue of *Experimental and Molecular Pathology* shows that serum anti-squalene antibodies were found in patients with the GWS-like illness who received anthrax vaccine known to be contaminated with squalene, a naturally-occurring lipid. These patients were all vaccinated after 1997, and the published data indicates that receipt of the contaminated vaccine was associated with the production of the antibodies.

In an earlier study, "Antibodies to Squalene in Gulf War Syndrome," which was published in the same journal in February 2000, antibodies to squalene were found in GWS patients who were veterans of the 1990-1991 Persian Gulf war. The presence of these antibodies in both groups of patients strongly suggests that the GWS-like illness experienced by the post-1997 patients is actually GWS, and, further, that GWS is not related to any event which took place in the 1990-1991 Persian Gulf theater. Contaminated vaccine lots might therefore account for the 1990-1991 GWS cases as well as for the post-1997 cases.

Neither study suggests how the vaccine may have become contaminated with squalene. Squalene is found in humans, animals and plants, and squalene from shark oil and other sources has been used for many years as an ingredient in cosmetics and other personal care preparations. It is possible that the small amounts of squalene found in the contaminated post-1997 vaccine lots could have been accidentally introduced during the production process. Squalene has been used as an adjuvant in several experimental human vaccines, including HIV vaccines, in efforts to boost the immune response, but the DoD has stated in the past that squalene was never intentionally added to the anthrax vaccine.

To determine if a vaccine did cause an illness, epidemiologists would conduct a study of the exposures of the ill individuals to the vaccine and to all other possible causative agents. An epidemiological study of non-U.S. Gulf War soldiers published in The British Medical Journal in 2000 (Hotopf et al) found a significant association between vaccinations and ill health. Incomplete U.S. medical records have made it impossible to adequately determine the vaccine exposures of many U.S. GWS patients, so an epidemiological study to examine whether certain lots of the anthrax vaccine cause GWS in U.S.

troops cannot easily be conducted by the DoD. The presence of anti-squalene antibodies appears to be a useful laboratory marker for use in identifying and characterizing GWS patients, and, in the absence of epidemiological data, the anti-squalene antibody test may also prove to be useful in studying the origins of GWS.

The announcement of the August 2002 publication was made by Autoimmune Technologies LLC, a New Orleans biomedical company. The research described in both the August 2002 article and the February 2000 article makes use of the anti-squalene antibody test. The antibodies were discovered by Dr. Robert F. Garry, a professor of microbiology and immunology at Tulane University Medical School who developed the test. Tulane has licensed the anti-squalene antibody technology to Autoimmune Technologies. Dr. Garry is the senior author of the August 2002 article.

U.S. Army researchers confirmed the discovery of the antibodies with their own version of the anti-squalene antibody test and published their work in November 2000. U.S. Patent No. 6,214,566 covering the anti-squalene antibody test was awarded to Tulane several months later, in April 2001. Because the testing method used by the Army researchers is covered by the Tulane patent, Autoimmune has recently offered the test to the DoD so that the DoD can sponsor a confirmatory study of all of the published data.

The August 2002 study looked at individuals who had participated in the DoD's Anthrax Vaccine Immunization Program, or AVIP. The DoD initiated the AVIP in 1997 to immunize 2.4 million military personnel considered to be at risk for exposure to anthrax. The U.S. Food and Drug Administration (FDA) subsequently assayed the AVIP anthrax vaccine for squalene content and found that five lots of the vaccine tested positive for small amounts of squalene. Some individuals participating in AVIP reported adverse vaccine reactions with GWS-like symptoms, so, because of the symptom similarities, the researchers tested several groups of AVIP participants for the presence of anti-squalene antibodies.

The researchers initially tested serum samples from six AVIP vaccine recipients who exhibited GWS-like symptoms and found that all six were positive for anti-squalene antibodies. All of these individuals had received inoculations from lots of the vaccine shown by the FDA to contain squalene.

The researchers conducted further blinded tests with 25 AVIP vaccine recipients plus 19 control individuals who did not receive the vaccine and did not have GWS symptoms. Of the 25 vaccine recipients, 17 had received vaccine from the five squalene-containing lots. Eight of these 17 individuals tested positive for anti-squalene antibodies, while none of the individuals who had received vaccine from other lots tested positive, a statistically significant difference ($p<0.025$). In addition, of the total of 20 AVIP vaccine recipients who tested positive for the antibodies in the study, 19 received vaccine from the squalene-containing lots. These results suggest that vaccination with the squalene-containing vaccine lots is associated with the production of the antibodies.

Of the 19 controls in the blinded study, only three tested positive for anti-squalene antibodies. All three of these individuals had undergone major surgery in the past, and they were the only members of the control group to have done so. The study presented no data that might explain this phenomenon, though it is possible that cell damage associated with major surgery exposes the surgery patients to immunologically significant amounts of intracellular squalene.

The researchers also conducted time-related tests which included four AVIP-vaccinated individuals for whom both pre-vaccination and post-vaccination sera were available. The post-vaccination sera from three of these four individuals tested positive for anti-squalene antibodies, while the pre-vaccination sera from all four individuals tested negative.

The article published in February 2000 looked at 1990-1991-era patients instead of AVIP participants. This article included a blinded study which showed that anti-squalene antibodies were found in 36 of 38 GWS patients who had been deployed to the Persian Gulf theater, in all 6 of the 6 GWS patients who had not been deployed to the Persian Gulf theater, and in none of the 12 control subjects who had been deployed but were not ill.

Data from two patients who had participated in a vaccine trial involving squalene was also discussed in the February 2000 article. After receiving a vaccine to which squalene had been added as an experimental adjuvant, both of these patients developed a multi-symptom disorder similar to GWS. Both tested positive for anti-squalene antibodies, though this sample of two patients was too small to determine the statistical significance, if any, of this finding.

GWS is usually difficult to differentiate from other rheumatic disorders, many of which have similar symptoms. Before the anti-squalene antibody test was developed, there was no specific laboratory test for GWS. Both articles suggest that the antibodies can serve as an excellent laboratory marker for GWS, since none of the control subjects in either published study tested positive for the antibodies except the three surgery patients in the August 2002 study. Using the antibodies as a laboratory marker for GWS could be very useful in helping physicians diagnose the disorder and in differentiating it from other rheumatic illnesses.

Anti-squalene antibodies might also provide a key to more effectively treating GWS patients. The presence of the antibodies in GWS patients indicates that the immune system is involved in the development of GWS. Effective drugs which modulate the human immune system are already in wide use, but they have not been previously considered to be appropriate for GWS patients. The published data now suggests that the use of immune modulators in GWS patients should be studied.

Prior to the publication of the February 2000 article, representatives of the U. S. General Accounting Office (the GAO) visited with Dr. Garry, reviewed the laboratory methods used in the anti-squalene antibody test, and discussed the initial data obtained by testing GWS patients. The GAO representatives determined that the test methodology was sound and that the patient data appeared compelling. On March 29, 1999, the GAO issued Report No. GAO/NSIAD-99-5, entitled "Gulf War Illnesses - Questions About the Presence of Squalene Antibodies Can Be Resolved." This report is available at: http://frwebgate.access.gpo.gov/cgi-bin/useftp.cgi?IPaddress=162.140.64.88&filename=ns99005.pdf&directory=/diskb/wais/data/gao

The GAO report urged the DoD to conduct its own research into anti-squalene antibodies with two objectives in mind: (1) to confirm the existence of the newly-discovered antibodies, and (2) to acquire patient data, explore the apparent link between the antibodies and the illness in GWS patients, and attempt to confirm or disprove the existence of such a link.

To satisfy the first GAO objective, the Army researchers confirmed that anti-squalene antibodies do indeed exist and can reliably be detected. They published their findings in an article entitled "Induction and Detection of Antibodies to Squalene" which appeared in the November 2000 issue of the *Journal of Immunological Methods.* The Army researchers conducted their testing by applying squalene to the wells of ELISA plates, and Dr. Garry and his colleagues conducted their testing by applying squalene to nitrocellulose strips in a Western-blot-type assay. There is no material difference between the two test methods.

Although the Army researchers confirmed the validity of the test and thus added support to the February 2000 patient data, their November 2000 article included no patient data of its own and as a

result did not specifically address the GAO's second objective. The Army researchers also failed to embrace the peer-reviewed February 2000 data itself, as is discussed in the statement Dr. Garry submitted to the House Subcommittee on National Security, Veterans Affairs and International Relations for the record of its hearing into Gulf War illnesses on January 24, 2002.

Dr. Garry pointed out that neither the February 2000 study nor the August 2002 study included large numbers of patients, and he also noted that both studies made use of self-selected volunteers, which were the only subjects available to the researchers. "Large studies can sometimes reveal relationships not seen in smaller studies, and studies which use pre-defined inclusion and exclusion criteria to recruit participants are preferred because they are less likely to be subject to possible self-selection biases," Dr. Garry said. "But the published data is so compelling as to transcend these minor shortcomings."

"A large confirmatory study of subjects who are not self-selected appears to us to be the only appropriate step at hand, and we are urging the DoD to sponsor such a study," said Dr. Russell B. Wilson, president of Autoimmune and another author of the August 2002 article. "We believe that an investigation into the relationship between anti-squalene antibodies and GWS will lead to a better understanding of the illness and ultimately to more effective treatments for the patients who have it," he said.

Although it maintains a waiting list of GWS patients and their physicians who may be interested in having the anti-squalene antibody test run for investigational use, the company has not yet made the test generally available.

For further information, please visit www.autoimmune.com.

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